

-continued

HPL Concentration ng/ml	OD ₄₆₀	% Color Bound
1000	0.78	35

Unknown samples could be assayed using the standard curve plotted from these data, as described in Example 1c.

EXAMPLE 7

This Example relates to the use of the present method and device in the determination of hepatitis B antigen wherein a direct radioimmunoassay technique is followed.

a. Production of hepatitis B antibodies

Anteserum containing hepatitis B antibodies were obtained following the procedure described in *The Journal of Immunology*, Vol. 109, No. 4 at Page 835.

b. Preparation of the test device

CNBr-activated Sepharose 4B, available from Pharmacia AB, Uppsala, Sweden, was swollen in 0.001M HCl. The antibodies contained in the antiserum prepared according to procedure *a.* above were coupled to the swollen CNBr-activated Sepharose 4B by mixing the antiserum with the Sepharose gel slurry in the proportions of 5-20 mg protein per gram of activated Sepharose 4B for 1 hour at 25° C. The resulting mixture was stirred for 17 hours at 4° C. The antibody-coupled Sepharose suspension was alternately washed with a buffer containing 0.1M tris (hydroxymethyl) aminomethane-0.5M NaCl adjusted to pH 8.2 with HCl and a buffer containing 0.1M sodium acetate-0.5 NaCl adjusted to pH 4.0 with acetic acid. The washed antibody-coupled Sepharose was slurried in tris (hydroxymethyl) aminomethane to give a 50% Sepharose suspension and 1-2 ml of this slurry was added to 3 ml plastic Stylex syringes, available from Pharmaseal Laboratories, Glendale, California, fitted with polyethylene support disks.

c. Performance of the assay

Sera which was either positive or negative with respect to the presence of hepatitis B antigen were added to the columns prepared according to procedure *b.* above in 0.1 ml volumes and allowed to incubate for 30 minutes. The reference sample containing hepatitis B antibody labeled with ¹²⁵I according to the procedure described in *The Journal of Immunology*, Vol. 109, No. 4 at Page 835 was then added to each column in 0.1 ml portions and allowed to incubate for 1½ hours. The columns were washed with 25 ml of buffer containing 0.1M tris (hydroxymethyl) aminomethane -0.5 NaCl-0.01% Tween 20 (a non-ionic surfactant available from the Atlas Powder Co., Wilmington, Delaware), and then counted using a Gammacord gamma counter. It was found that about 12 times more labeled antibody was retained in the columns that were contacted with positive sera than those contacted with negative sera.

What is claimed is:

1. A method for the quantitative determination of a specific binding substance which method comprises the steps of:

- a. contacting a matrix contained in a column with a predetermined quantity of a liquid sample containing said substance to be determined and with a predetermined quantity of a reference sample con-

taining a labeled form of said substance to be determined or a specific binding partner thereto, said matrix being porous and insoluble with respect to said liquid sample and said reference sample and having specific binding partners to said substance to be determined immobilized therewith;

- b. contacting said matrix with a liquid capable of eluting from said matrix substantially all of the labeling component originating from the reference sample and not bound to the specific binding partners immobilized with said matrix a predetermined period of time after step (a); and

c. determining the relative amount of said labeled component which is retained in said column, which relative amount is a function of the amount of said substance to be determined in said liquid sample.

2. A method as in claim 1 wherein step (a) is accomplished by contacting said matrix with a mixture comprising a predetermined quantity of said liquid sample and a predetermined quantity of said reference sample, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of both the substance to be determined in the liquid sample and the labeled component in the reference sample in the time that the mixture and the matrix are in contact prior to step (b).

3. A method as in claim 2 wherein said contact between said matrix and said mixture is prolonged for a predetermined incubation period.

4. A method as in claim 3 wherein said incubation period ranges from 15 minutes to 12 hours.

5. A method as in claim 1 wherein said labeled form is of said substance being determined and wherein step (a) is accomplished by:

- (1) contacting said matrix with a predetermined quantity of said liquid sample so that some of the specific binding partners immobilized with the matrix remain unbound, and

(a) (2) thereafter contacting said matrix with a predetermined quantity of said reference sample, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of the substance to be determined in said predetermined quantity of fluid sample contacted with said matrix in step (a) (1) in the time that the predetermined quantity of fluid sample and the matrix are in contact prior to step (a) (2), and the amount of labeled component in said predetermined quantity of reference sample contacted with said matrix in step (a) (2) being sufficient to bind a portion or all of the remaining unbound immobilized specific binding partners in the time that said predetermined quantity of reference sample and said matrix are in contact prior to step (b).

6. A method as in claim 5 wherein said contact between said matrix and said predetermined quantity of liquid sample and said contact between said matrix and said predetermined quantity of reference sample are prolonged for predetermined incubation periods which may be the same or different.

7. A method as in claim 6 wherein said predetermined incubation periods range between 15 minutes and 12 hours.

8. A method as in claim 5 which comprises the additional step between steps (a)(1) and (a)(2) of contacting said matrix with an eluting liquid capable of eluting